09/788,286

WEST Search History

DATE: Friday, November 15, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L18	L17 same l15	1	L18
L17	isotopically	2127	L17
L16	L15 same l2	0	L16
L15	L14 not l7	31	L15
L14	L13 same l12	32	L14
L13	phosphorylat$	24874	L13
L12	L11 same (l10 or l9)	205	L12
L11	mass adj spectrometry	19239	L11
L10	threonine or serine or tyrosine	54986	L10
L9	phosphoserine or phosphothreonine or phosphotyrosine	2762	L9
L8	threonine or serine or typrosine	39468	L8
L7	l2 adj2 l3 adj l4 adj L5	18	L7
L6	l2 adj2 l3 adj l4adj L5	76430	L6
L5	tag	76430	L5
L4	affinity	116828	L4
L3	coded	143790	L3
L2	isotope	28268	L2
L1	ICAT	127	L1

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L18: Entry 1 of 1 File: PGPB Aug 29, 2002

PGPUB-DOCUMENT-NUMBER: 20020119505
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020119505 A1

TITLE: Phosphoprotein binding agents and methods of their use

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 788286 [PALM]
DATE FILED: February 16, 2001

INT-CL: [07] G01 N 33/537, G01 N 33/543

US-CL-PUBLISHED: 435/7.92
US-CL-CURRENT: 435/7.92

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The invention provides reagents and methods for characterizing (i.e., identification and/or quantitation) the phosphorylation states of proteins. Proteins may be post-transcriptionally modified such that they contain phosphate groups at either some or all of their serine, threonine, tyrosine, histidine, and/or lysine amino acid residues. In many cases the extent to which a protein is phosphorylated determines it bioactivity, i.e., its ability to effect cell functions such as differentiation, division, and metabolism. Hence, a powerful tool for diagnosing various diseases and for furthering the understanding of protein-protein interactions is provided.

Please return all attachments with search results. Thanks.

LB

Access DB# 80268

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Molly Ceperley Examiner #: 59757 Date: 11/15/02

Art Unit: 1641 Phone Number 30 8-4239 Serial Number: 09/788286

Mail Box and Bldg/Room Location: → CM1-5D15 Results Format Preferred (circle): PAPER DISK E-MAIL

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Phosphoprotein Binding Agents and methods of use

Inventors (please provide full names): Michael R. Goshe, Thomas P. Conrads, Timothy D. Veenstra

Earliest Priority Filing Date: 02/16/01

For Sequence Searches Only *Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

Please search for the method of claim 1 using the following terms:

phosphorylat?
dephosphorylat?
threonine
serine
tyrosine
phosphothreonine
phosphoserine
phosphotyrosine
phosphate reactive group
1,2-ethanedithiol (EDT)
alkyne dithiol
Michael Addition (reacts EDT)

mass spectrometry
differentially isotopically labeled
affinity label (capture reagent)
heavy isotopes (^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{34}S)
isotope-coded affinity tag (ICAT)

C. Chan

Rush

See all claims attached.

Point of Contact:
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Searcher: Thomas G. Larson, Ph.D.
Searcher Phone #: 703-308-7309
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Date Searcher Picked Up: 11/20/02
Date Completed: 11/21/02
Searcher Prep & Review Time: 60
Clerical Prep Time:
Online Time: 275

Type of Search

NA Sequence (#)
AA Sequence (#)
Structure (#)
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Other (specify)

PTO-1590 (8-01)

What is claimed is:

1. A method of comparing the phosphorylation states of one or more proteins in two or more samples comprising:

providing a substantially chemically identical and differentially isotopically labeled protein reactive reagent for each sample wherein the protein reactive reagent satisfies the formula:

B-L-PhRG

wherein B is a binding agent that selectively binds to a capture reagent (CR), L is a linker group having one or more atoms that are differentially labeled with one or more stable isotopes, and PhRG is a phosphate reactive group that selectively reacts with amino acid residues that were formerly phosphorylated;

reacting each sample with one of the protein reactive reagents to provide proteins bound to the protein reactive reagent, whereby such bound proteins are differentially labeled with stable isotopes; → (ICAT ?)

capturing bound proteins of the samples using the capture reagent that selectively binds the binding agent;

releasing captured bound proteins from the capture reagent by disrupting the interaction between the binding agent and the capture reagent; and

detecting the released bound proteins.

2. The method of claim 1, wherein the bound proteins in the samples are enzymatically or chemically processed to convert them into bound peptides.

3. The method of claim 1, wherein a protein portion of one or more of the bound proteins are sequenced by tandem mass spectrometry to identify the bound protein.

4. The method of claim 1, wherein the amount of one or more phosphorylated proteins in the sample is determined by mass spectrometry and further comprising introducing into a sample a known amount of one or more internal standards for each protein to be quantified.

5. The method of claim 1, wherein one or more phosphorylated amino acid residues are selected from the group consisting of threonine, serine, and tyrosine.

6. The method ~~of claim 1,~~ wherein the released bound proteins are separated by chromatography prior to detecting the bound proteins by mass spectrometry.

7. The method of claim 1, wherein a plurality of proteins in a single sample are detected and identified.

8. The method of claim 3, wherein all of the proteins in a sample are identified.

9. The method of claim 1, wherein relative amounts of one or more proteins in two or more samples are determined and further comprising combining differentially labeled samples, capturing bound proteins from the combined samples and measuring relative abundances of the bound proteins differentially labeled proteins.

10. The method ~~of claim~~ 1, wherein the proteins being quantified are membrane proteins.

11. The method of claim 1, wherein different samples contain proteins originating from different organelles or different subcellular fractions.

12. The method of claim 9, wherein different samples represent proteins expressed in response to different environmental or nutritional conditions, different chemical or physical stimuli or at different times.

13. The method ~~of claim~~ 1, wherein the different samples represent proteins expressed in different disease states.

14. A method for screening for a therapeutic that alters a phosphorylation state of a protein, the method comprising:

contacting at least one test sample containing the protein with the therapeutic;

providing at least one control sample containing the protein;

removing one or more phosphate groups from one or more amino acid residues of the protein in the at least one test sample and the at least one control sample;

tagging the at least one test sample and the at least one control sample with substantially chemically identical and differentially isotopically labeled protein reactive reagents for each sample, wherein the protein reactive reagents satisfies the formula:

B-L-PhRG

wherein B is a binding agent that selectively binds to a capture reagent, L is a linker group having one or more atoms that are differentially labeled with one or more stable isotopes, and PhRG is a phosphate reactive group that selectively reacts with amino acid residues that were formerly phosphorylated; and

detecting a level of phosphorylation of the tagged proteins in the at least one test sample and the at least one control sample; and

determining whether the therapeutic altered the level of phosphorylation of the tagged proteins in the at least one test sample.

15. A reagent for mass spectrometric analysis of proteins that satisfies the general formula:

B-L-PhRG

where B is a binding agent that selectively binds to a capture reagent, L is a linker group that comprises at least one isotopically heavy atom and a phosphorylation reactive group (PhRG) that selectively labels proteins at one or more residues that were formerly occupied by phosphate groups.

16. The reagent of claim 15, wherein PhRG is selected from the group consisting essentially of primary amines, secondary amines, tertiary amines,